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Elimination of *Arsenophonus* and decrease in the bacterial symbionts diversity by antibiotic treatment leads to increase in fitness of whitefly, *Bemisia tabaci*

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ABSTRACT

Bemisia tabaci is an invasive agricultural pest with more than 24 genetic groups harboring different bacterial endosymbionts categorized into obligatory and facultative endosymbionts. *Arsenophonus* is one of the facultative endosymbionts prevalent in *B. tabaci* of Indian sub-continent. Not much is known about the functional role of this endosymbiont in its host. Some studies have revealed its involvement in virus transmission by *B. tabaci*, but how it effects the biology of *B. tabaci* is unknown. In this study, tetracycline was used to eliminate *Arsenophonus* from *B. tabaci* to study its effects with regard to development and other fitness parameters. Bacteria specific 16S Polymerase chain reaction (PCR) was used to ascertain *Arsenophonus* absence with differential effects on other secondary endosymbionts present in *B. tabaci*. Our results revealed that *Arsenophonus* negative (A^-) whiteflies had more fecundity, increased juvenile developmental time, increased nymphal survival and increased adult life span as compared to control (A^+) whiteflies. Thus, our results demonstrate that A^+ whiteflies have lesser fitness as compared to A^- whiteflies. These observations give a new insight about the probable role of *Arsenophonus* in *B. tabaci*, that need to be explored further.

1. Introduction

Bemisia tabaci (Gennadius), (Homoptera: Aleyrodidae) is a worldwide pest of agricultural crops and ornamental plants (Ahmed et al., 2009). They feed exclusively on phloem sap that is rich in carbohydrates and lacking in essential amino acids. The absence of essential amino acids in diet is suggested to be ameliorated by endosymbionts in these insects (Buchner, 1965; Su et al., 2013; Solan and Moran, 2012). Endosymbionts in insects have been categorized into two types- primary endosymbionts and secondary endosymbionts. Primary or obligatory endosymbionts found in bacteriocytes (Moran and Telang, 1998) are vertically transmitted and are thought to synthesize essential non-dietary metabolites (Clark et al., 2010; Douglas, 2009). The secondary or facultative endosymbionts are also vertically transmitted from mother to offspring and can also be transmitted horizontally through direct or indirect contact with other infected individuals (Clark et al., 2010; Feldhaar, 2011; Baumann et al., 2006). Although the role of different facultative bacterial endosymbionts in *B. tabaci* is largely unknown, there are evidences which point toward their contribution to the biological differences observed among different genetic groups (Gueguen et al., 2010) with some having competence to act as reproductive manipulators of their host (Moran et al., 2008; Feldhaar, 2011). In fact, different combinations of secondary endosymbionts have been associated with different genetic groups of whiteflies (Biotypes- Q and B) (Chiel et al., 2007; Costa et al., 1995). Though, the biological contribution of bacterial endosymbiont community to their host, *B. tabaci* has been well explored (Xue et al., 2012; Himler et al., 2011; Su et al., 2013; Fang et al., 2014), the importance to the host of specific endosymbionts has not been established.

Portiera aleyrodidarum is the only primary endosymbiont of whitefly (Baumann, 2005), while secondary endosymbionts include a range of bacteria like *Wolbachia* (Rickettsiales); (Zchori-fein and Brown, 2002), *Arsenophonus* (Enterobacteriales); (Thao

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and Baumann, 2004), *Cardinium* (Bacteroidetes); (Weeks et al., 2003), *Rickettsia* (Rickettsiales); (Gottlieb et al., 2006), *Hamiltonella* (Enterobacteriales); (Zchori-fein and Brown, 2002) and *Fritschea* (Chlamydiales); (Everett et al., 2005). Different genetic groups of *B. tabaci* have been known to carry different secondary endosymbionts. Endosymbionts have a variety of effects on their hosts such as increasing tolerance to heat stress (Montllor et al., 2002), increasing resistance to parasitic wasps (Oliver et al., 2005) and causing host plant specialization (Tsuchida et al., 2004). In fact, several secondary endosymbionts appear to affect the capability of the host insect to be a pest. *Hamiltonella* and *Arsenophonus* have been described to have significant contribution in virus transmission in plants (Gottlieb et al., 2010; Rana et al., 2012). Role of *Rickettsia* in heat tolerance and increased susceptibility to some insecticides like imidacloprid, thiamethoxam and pyriproxyfen is well documented (Brumin et al., 2011; Kontsedalov et al., 2008). It was also reported to play an important role in increasing fecundity, survival to adulthood and reduction in development time (Himler et al., 2011).

However, the full range of functions of different endosymbionts have not been extensively studied and the relationship between *B. tabaci* and its endosymbionts is partly understood because of the technical difficulty in culturing most of the endosymbionts *in vitro*. The role of endosymbionts in whitefly may be determined if they could be removed *in vivo* by using specific antibiotics. Different studies have assessed the effect of different antibiotics on different bacterial endosymbionts. Ruan et al. (2006), studied the effect of three antibiotics – tetracycline, ampicillin trihydrate and rifampicin on the fitness of different genetic groups of *B. tabaci*. Their results confirmed the removal of three secondary endosymbionts *Arsenophonus*, *Hamiltonella* and *Wolbachia*. Ahmed et al. (2010), showed *in vivo* sensitivity of endosymbionts in three genetic groups (biotypes- B, Q and Cv) to ampicillin, rifampicin and tetracycline, where *P. aleyrodidarum* was found to be unaffected by these treatments while the antibiotics had substantial effect on secondary endosymbionts. However, the 3 genetic groups showed different responses to different antibiotics. Xue et al. (2012), showed elimination of *Wolbachia* in *B. tabaci* Mediterranean with rifampicin that inturn affected whitefly development and reproduction. Thus, these studies confirmed the elimination of secondary endosymbionts by use of specific antibiotics which further helped in studying the role of bacterial endosymbionts. Keeping in view the literature, we used this approach of removing secondary endosymbiont *Arsenophonus* in *B. tabaci* and studied the functional role of this bacterium by comparing the life history traits of treated (A^-) and untreated (A^+) whiteflies.

2. Material and methods

2.1. Whitefly culture

B. tabaci was collected from cotton plant from the fields of Indian Agricultural Research Institute, Delhi and then maintained in insect proof climate control chambers. The population was reared on cotton at 27 ± 2 °C, photoperiod of 14:10 h (L:D) and 60–70% relative humidity. The genetic group was identified using mitochondrial cytochrome oxidize 1 (mtCO1) gene markers (Singh et al., 2012).

2.2. DNA extraction from B. tabaci for detection of endosymbionts

Individual whiteflies were used for DNA isolation. The whiteflies were washed in 200 μL of autoclaved water and then homogenized with the help of hand held homogenizer (Pellet pestles cordless motor, SIGMA–ALDRICH, Z359971-1EA) in 14 μL of lysis buffer (100 mM Tris–Cl pH 8.0, 1% SDS, 100 mM NaCl and 100 mM EDTA pH-8, 1%). 2 μL of Proteinase K (0.28 μg/μL; SIGMA–ALDRICH Catalog No. 39450-01-6) was added in the homogenized mixture and incubated at 65 °C for 45 min. After incubation 20 μL of pre-chilled 5 M potassium acetate and 8 μL of 6 M lithium chloride was added in the incubated homogenate and kept in ice for 15 min. The mixture was centrifuged at 10,000 rpm for 15 min. After centrifugation, the supernatant was taken and 0.6 volume of isopropanol was added. The supernatant and isopropanol mixture was again centrifuged at 10,000 rpm for 15 min. The pellet obtained after centrifugation was washed in 70% ethanol. After ethanol wash, the pellet was air dried and dissolved in elution buffer (10 mM Tris–Cl, pH 8.0) followed by RNase (0.1 μg/μL) treatment for 45 min at 37 °C. The DNA was then checked on 0.5% agarose gel containing ethidium bromide (0.5 μg/mL) by running it at 110 volts for 15 min and then observed using UV transilluminator (FOTODYNE incorporated, USA).

2.3. Diagnostic PCR for detection of bacterial endosymbionts in B. tabaci population

B. tabaci population was taken from insect proof climate control chambers at IARI, Pusa, Delhi. Fifty whiteflies were collected randomly for the experiment and diagnosed for the presence of different bacterial endosymbionts- *Portiera*, *Wolbachia*, *Rickettsia*, *Arsenophonus* and *Cardinium*. Specific bacterial primers were used for amplification of 16S rRNA bacterial gene (Table 1). For each bacterial endosymbiont, PCR mix consisting of dNTPs (2.5 mM), 1× buffer (2.5 μL), Taq polymerase (1 U), Forward and Reverse primers (7.5 pmol each), DNA template (25–30 ng) was prepared and the final volume was raised up to 25 μL with autoclaved mili-Q (MQ) water (Millipore corporation water purifying system).

PCR conditions involved denaturation at 94 °C for 30 s and annealing at different temperatures specific for each bacterial endosymbiont (*Portiera* 58 °C, *Wolbachia* 52 °C, *Arsenophonus* 55 °C, *Rickettsia* 55 °C, *Cardinium* 50 °C) for 30 s. Extension was carried out at 72 °C for 40 s with the final extension for 5 min at 72 °C. Forty-five number of cycles were fixed for each bacterial endosymbiont detection.

The plasmids containing 16S rRNA gene of different bacterial endosymbiont were used as positive controls while the reaction

Table 1
Primers and PCR cycling conditions for the identification of bacterial endosymbionts associated with *B. tabaci*.

Endosymbiont	Primer sequence	PCR cycle	Annealing temp	Product size	References
Portiera	F-5′TGCAAGTCGCGGCATCAT3′ R-5′CCGCCTTCTGCGTTGGCAACT3′	45	58 °C	1000 bp	Singh (2013)
Wolbachia	F-5′CGGGGGAAAATTTATTGCT3′ R-5′AGCTGTAATACAGAAAGGAAA3′	45	52 °C	650 bp	Singh et al. (2013)
Rickettsia	F-5′GCTCAGAACGAACGCTGG3′ R-5′GAAGGAAAGCATCTCTGC3′	45	55 °C	800 bp	Gottlieb et al. (2006)
Arsenophonus	F-5′CGTTTGATGAATTCATAGTCAAA3′ R-5′GGTCCTCCAGTTAGTGTTACCCAAC3′	45	52 °C	630 bp	Singh et al. (2013)
Cardinium	F-5′GCGGTGTAAAATGAGCTTG3′ R-5′ACCTCTTCTTTAACTCAAGCCT3′	45	50 °C	440 bp	Weeks et al. (2003)

without any DNA was used as negative control for each reaction. *Portiera* being the primary endosymbiont was expected to be present in all the samples. The PCR product was then checked on 1% agarose gel. The PCR products for different bacteria exhibited sizes of different band length (*Portiera* 1000 bp, *Wolbachia* 650 bp, *Arsenophonus* 630 bp, *Rickettsia* 800 bp, *Cardinium* 440 bp).

2.4. Treatment of whiteflies with antibiotic tetracycline for endosymbiont elimination

2.4.1. Feeding chambers

Parafilm membrane sachets were used for direct feeding of newly emerged whitefly adults. Cotton leaves with 4th instar nymphs having red eyes were taken from the plants and inserted into the agar in petridish for maintaining rigidity and preventing wilting of leaf. The next day (at 9:00 A.M.), the newly enclosed adults were collected and sexed using stereomicroscope (Nikon 77345) and then put for feeding. Small cages made of plastic transparent sheets with 2.5 cm diameter and 2.5 cm height, sealed at one end were used. The newly emerged whiteflies were released into the cage from above and covered with stretched parafilm over which the feeding solution was placed. The feeding solution was again covered with another stretched parafilm.

2.4.2. Tetracycline treatment

The feeding solution contained 20% sucrose and 50 μL/mL of tetracycline (Himedia, Lot: 0000182187) in autoclaved MQ water. The whiteflies were allowed to feed at 27 ± 2 °C, photoperiod of 14:10 h (L:D) and 60–70% relative humidity for 48 h. The control was also used which included feeding solution without tetracycline. There were five replicates each for tetracycline treatment and for control, each replicate had 10 whiteflies for endosymbiont detection. PCR based detection analysis was performed which confirmed complete elimination of *Arsenophonus*, though *Wolbachia* and *Rickettsia* were not eliminated completely. Hence, we decided to look into the effects of *Arsenophonus* removal on *B. tabaci*.

2.5. Transfer of tetracycline fed and control whiteflies on plant for observation of developmental parameters

Five pairs of newly emerged adults of *B. tabaci* were fed tetracycline for 48 h and then transferred to clip cages on cotton plants along with controls. To determine the duration of different developmental stages, males and females were confined on the abaxial surface of a cotton leaves in clip-cages, as described by Zang et al. (2005), and Xu et al. (2010), with some modifications. The size of the cages was 3.5 cm in diameter and 2.5 cm in height. The experiment was also performed at 27 ± 2 °C with photoperiod of 14:10 h (L:D) and 60–70% relative humidity. The eggs laid and different life-cycle stages were observed at 24 h intervals with Leica ES2 stereozoom microscope. The F1 adults that emerged were anaesthetized with carbon dioxide and sexed for calculating sex ratio.

2.5.1. Assessment of fitness parameters

Fecundity, fertility (hatchability rate), adult longevity, developmental period (from egg to adult emergence) of F1 juveniles, F1 progeny sex ratio, nymphal survival percentage [(Total number of adults emerged/total number of eggs hatched) × 100] were recorded for control and treated individuals. The fecundity, fertility (hatchability rate) and survival data were recorded on a daily basis till the end of the experiment.

2.6. Data analysis

Data were subjected to separate mixed-model analyses of variance (ANOVA, Statistica 12, Statsoft, Inc. Tulsa, USA), treating replication as a random factor and treatment as fixed factor crossed with replication. In all cases treatment means were used as units of analysis. Graphical presentations of data are indicated as 'Mean ± SE'. Correlation analysis was performed to ascertain the effect of *Arsenophonus* elimination on different life history traits.

3. Results

3.1. Endosymbiont detection in B. tabaci population

The partial sequencing of mtCO1 markers revealed that the population used in this study belonged to the Asia II–1 genetic group. Diagnostic PCR confirmed the presence of primary endosymbiont *Portiera* and secondary endosymbionts *Wolbachia*, *Arsenophonus* and *Rickettsia* in this population. The infection rates for different bacterial endosymbionts are given in Fig. 1.

3.2. Effect of tetracycline treatment on bacterial endosymbionts

Diagnostic PCR confirmed the successful removal of *Arsenophonus* after 48 h of feeding on tetracycline (Fig. 1). However, tetracycline treatment did not have any effect on the primary endosymbiont *Portiera*. Secondary endosymbionts *Wolbachia* and *Rickettsia* were reduced significantly but not completely removed. The removal was 0% for *Portiera*, 39% for *Wolbachia* and 27% for *Rickettsia*, while it was 100% for *Arsenophonus*, showing substantial effect of tetracycline on *Arsenophonus*. The elimination of endosymbionts was also checked in F1 progeny that emerged from tetracycline treated whiteflies. Our results showed 100% presence of primary endosymbiont *Portiera* in all the samples and statistically non-significant infection levels of *Wolbachia* and *Rickettsia* among the progeny and parents of control and treated groups. However, *Arsenophonus* was not detected in any of the F1 progeny samples from each pair of treated parents as compared to the F1 offspring from control parents where 50% of the progenies from each pair of parents showed presence of *Arsenophonus* (Fig. 1). These differential results of infection among the F1 progeny of treated parents prompted us to conduct further investigations to understand the possible roles of *Arsenophonus* on the biology of *B. tabaci*.

3.3. Effect of Arsenophonus elimination on fecundity, egg hatching and sex-ratio of F1 progeny

Arsenophonus elimination in *B. tabaci* had significant effect on the number of eggs laid by the treated adults (Fig. 2A). The A^- whiteflies oviposited more number of eggs as compared to A^+ whiteflies (A^+: 69.4 ± 3.91; A^-: 115.2 ± 6.65; $F_{(1,8)} = 35.25$, $P = 0.0003$). The highest number of eggs laid by an A^- female was 134, while for A^+ female it was 77. The females began to lay eggs within 24 h of introduction on the leaves of cotton plant. The proportion of nymphs hatched from the eggs in both A^+ and A^- whiteflies did not differ significantly ($F_{(1,8)} = 2.27$, $P = 0.17$). The fertility (egg hatching) rates were 62.23 ± 3.66% and 55.68 ± 2.97% respectively for A^+ and A^- whiteflies (Fig. 2B). The egg hatching rates ranged from 52.78% to 66.23% for A^+ and from 45.08% to 63.33% for A^- whiteflies. Of the F1 progeny that developed to adults, the percentage of males and females produced did not differ significantly between A^+ and A^- whiteflies ($A^+♂$: 70.65 ± 2.70%; $A^-♂$: 67.57 ± 2.80%; $F_{(1,8)} = 0.62$, $P = 0.45$; $A^+♀$: 29.35 ± 2.71%; $A^-♀$:

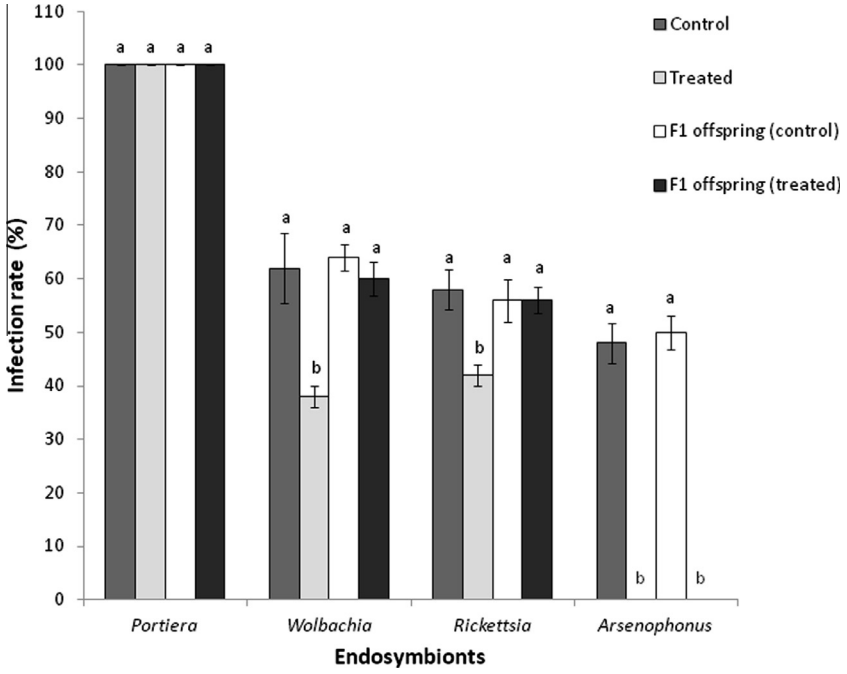

Fig. 1. Infection rates of different bacterial endosymbionts in control (A^+), treated (A^-), F1 offspring from treated and control whiteflies. Different alphabets over bars indicate significant difference at $P < 0.05$.

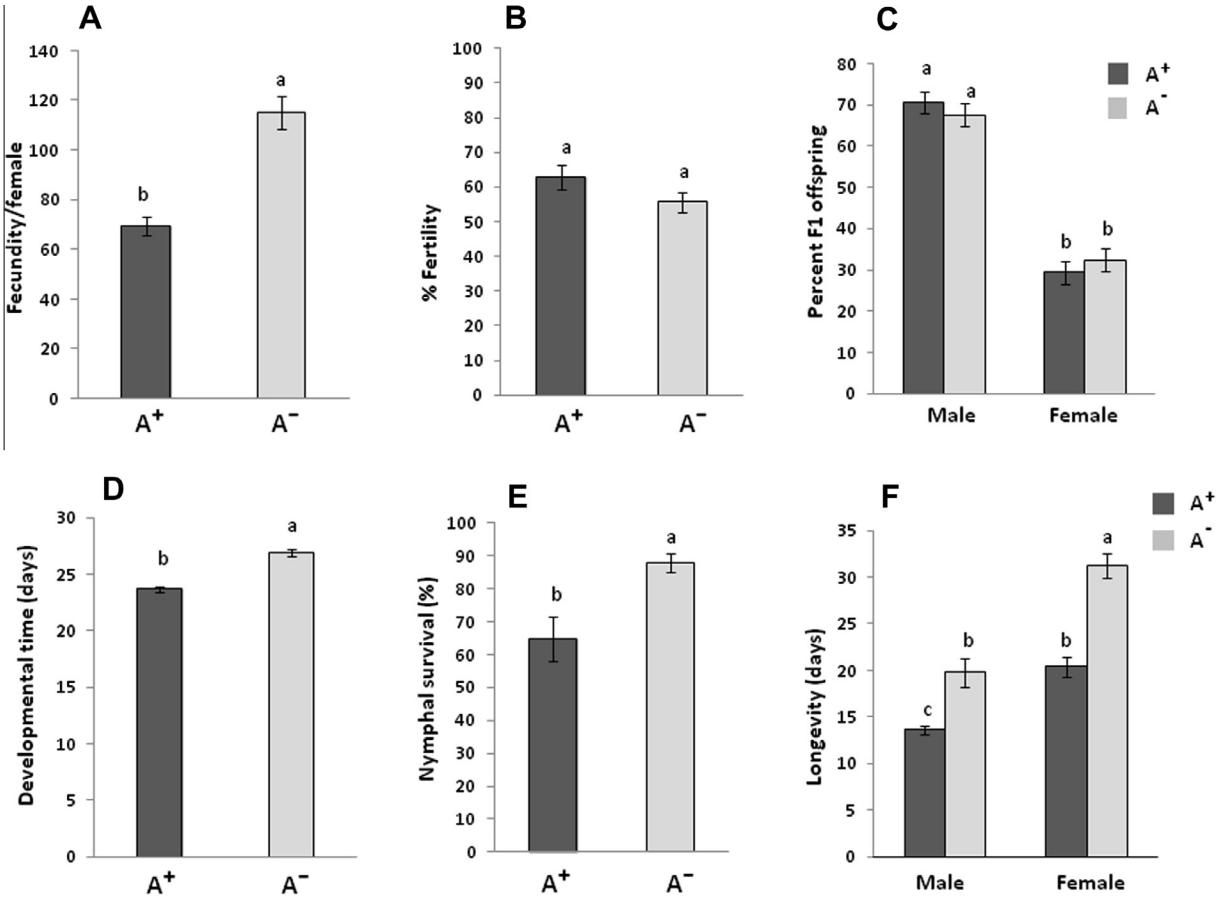

Fig. 2. Effects of *Arsenophonus* elimination on different life history traits of *B. tabaci*. (A) fecundity, (B) % fertility (hatchability rate), (C) percent survival of F1 males and females, (D) developmental time, (E) percentage survival of nymphs (F) longevity of adult male and female parents. Values are presented as mean ± SE and different letters above the columns indicate the significance difference at $P \leqslant 0.05$.

32.33 ± 2.77%; $F_{(1,8)} = 0.59$, $P = 0.46$). Therefore, the results indicate that antibiotic treatment had no significant effect on the sex-ratio of F1 progeny from A⁻ whitefly parents (Fig. 2C). The relationship between fecundity (eggs laid), fertility (hatchability rate), and the number of F1 adults emerged was assessed using correlation analysis. The analysis was performed on both A⁺ and A⁻ whiteflies. Though a significant correlation was detected between adults emerged and fecundity [slope (adults emerged vs fecundity) = 8.93 + 1.90, $r = 0.79$, $P = 0.05$], adults emerged and fertility (hatchability rate) [slope (adults emerged vs fertility) = −24.81 + 1.59, $r = 0.93$; $P = 0.01$] in A⁻ whiteflies, no significant correlation was detected between other combination of parameters in A⁺ and A⁻ whiteflies [A⁻ whiteflies: slope (fertility vs fecundity) = 55.51 + 0.93, $r = 0.62$, $P = 0.13$; A⁺ whiteflies: slope (adults emerged vs fecundity) = 68.33 + 0.038, $r = 0.03$, $P = 0.48$; slope (adults emerged vs fertility = 38.85 + 0.15, $r = 0.21$, $P = 0.36$; slope (fertility vs fecundity) = 34.68 + 0.80, $r = 0.47$, $P = 0.21$]. However, the *r*-values for different parameters for A⁻ whiteflies were on higher side as compared to the A⁺ whiteflies.

3.4. Effect of Arsenophonus elimination on development of B. tabaci juveniles

The egg to adult development time was significantly higher in the progeny of A⁻ parents exposed to tetracycline than progeny from A⁺ control parents (Fig. 2D; $F_{(1,8)} = 51.09$, $P = 0.0001$). The mean development time of progeny of control group was 23.68 ± 0.29 days while that of progeny of tetracycline fed parents was 26.94 ± 0.34 days. Further, the mean percentage of F1 juveniles that successfully completed development to adulthood was significantly higher among the progeny from A⁻ group (87.88 ± 2.98%) than those of A⁺ group (64.61 ± 6.73%) (Fig. 2E; $F_{(1,8)} = 9.99$, $P = 0.01$).

The stage specific developmental time also showed differences between the A⁺ and A⁻ whiteflies (Table 2). There were significant differences in egg incubation period ($F_{(1,8)} = 41.47$, $P = 0.0002$), second instar ($F_{(1,8)} = 27.38$, $P = 0.0008$) and fourth instar ($F_{(1,8)} = 16.13$, $P = 0.0038$), though there was no significant difference in first ($F_{(1,8)} = 0.69$, $P = 0.43$) and third ($F_{(1,8)} = 0.18$, $P = 0.89$) instar development time. The incubation period of eggs laid by A⁻ parents was 1.74 days more than the untreated control. Similarly, the development time for 2nd and 4th instars from A⁻ parents was 0.7 and 0.52 days longer than their respective controls.

3.5. Effect of Arsenophonus elimination on Longevity of treated B. tabaci

Elimination of *Arsenophonus* significantly affected the longevity of both A⁻ males and females (Fig. 2F). The average longevity of A⁻ males was 6.2 days more than that of untreated control (A⁺♂: 13.6 ± 0.51 days; A⁻♂: 19.9 ± 1.56 days; $F_{(1,8)} = 14.24$, $P = 0.005$). Similarly, A⁻ females survived 10.8 days longer than the A⁺ females (A⁺♀: 20.4 ± 1.08 days; A⁻♀: 31.2 ± 1.32 days; $F_{(1,8)} = 40.22$, $P = 0.0002$). In this experiment *Arsenophonus* was completely inactivated which indicates that the presence of *Arsenophonus* significantly reduces the longevity of the whiteflies. The longest individual male and female longevities for A⁺ and A⁻ whiteflies were 15, 24 days and 23, 33 days respectively.

4. Discussion

In this study, tetracycline treatment was able to completely eliminate *Arsenophonus* as confirmed by diagnostic PCR. For *Wolbachia* and *Rickettsia*, we got differential rates of elimination after tetracycline treatment. Moreover, the F1 progeny from tetracycline treated parents showed increased proportion of insects with *Wolbachia* and *Rickettsia* infection as compared to their tetracycline treated parents which may be due to buildup in density of these endosymbionts from the left over bacterial population. Such dose dependent relation of tetracycline and *Wolbachia* density has been shown in *Muscidifurax uniraptor* (Zchori-Fein et al., 2000). Different studies have been carried out where tetracycline has been reported to be effective in removal of *Arsenophonus* in *B. tabaci* (Ahmed et al., 2010; Ruan et al., 2006). However, the rate of elimination has been varying in different studies perhaps due to the different genetic group of the whitefly, the specific endosymbiont communities and their interactions.

Our results showed that *Arsenophonus* presence decreased the fecundity of females, nymphal survival and developmental time of nymphs. These results suggest that *Arsenophonus* infection reduces the fitness of whiteflies as indicated by a significant correlation between adults emerged vs fecundity and adults emerged vs fertility among the A⁻ group. Elimination of this bacterium within the hatched eggs may have led to emergence of increased number of progeny in A⁻ whiteflies. Moreover, the longevity of uninfected whiteflies both males and females increased as compared to those infected with *Arsenophonus*. These results correlate with earlier works by Ruan et al. (2006) and Fang et al. (2014) on other secondary endosymbionts *Hamiltonella* and *Cardinium* respectively wherein they have reported that secondary endosymbiont infection decreases the fitness and competitive ability of whitefly host. There have been other studies which have shown beneficial effects on growth, development and survival of whiteflies because of presence of specific secondary endosymbiont (Su et al., 2013; Xue et al., 2012; Himler et al., 2011; Costa et al., 1997). Our study also revealed that *Arsenophonus* is not involved in altering the progeny sex-ratio in Asia II genetic group of *B. tabaci* even though it has been shown to cause male-killing in parasitic wasps (Skinner, 1985; Werren et al., 1986; Gherna et al., 1991).

Arsenophonus has been reported to be associated with inducing different phenotypes in their hosts such as reproductive manipulation (son-killing) in parasitic wasp *Nosania* (Gherna et al., 1991), phytopathogenicity in sugar beet and strawberry by planthopper, *Cixiidae* (Danet et al., 2003; Bressan et al., 2008; Zreik et al., 1998) and virus transmission in *B. tabaci* (Rana et al., 2012). However, in the light of its symbiotic interactions with its

Table 2
Developmental periods of different stages of *B. tabaci* with respective *P*-values significant at $P \leqslant 0.05$.

	Developmental period (days, M ± SE)						
	n	Egg	1st instar	2nd instar	3rd instar	4th instar	Egg-adult
Control (A+)	50	5.32 ± 0.067	5.62 ± 0.069	4.72 ± 0.064	4.4 ± 0.07	3.64 ± 0.08	23.68 ± 0.299
Treated (A⁻)	50	7.06 ± 0.109	5.82 ± 0.089	5.42 ± 0.071	4.38 ± 0.069	4.16 ± 0.052	26.94 ± 0.344
P-value		0.0002	0.43	0.0008	0.89	0.0038	0.01

hosts and in view of recent insights on virus transmission by *B. tabaci*, the impact of *Arsenophonus* endosymbiont on the life-history traits of its host becomes important. Moreover, *Arsenophonus* has been found in wide range of genetic groups of whiteflies like Asia II 3 (ZHJ1 biotype), Asia II 7 (Cv biotype), Indian Ocean (Ms. biotype), Mediterranean, Sub-Saharan Africa species, Asia II 1, Asia I genetic groups (Gueguen et al., 2010; Chiel et al., 2007; Ahmed et al., 2009, 2010; Thierry et al., 2011; Singh et al., 2012), which further makes the study of this endosymbiont more important.

The removal of secondary endosymbionts from *B. tabaci* may produce both favorable and unfavorable effects on the fitness of the host insect (Ruan et al., 2006). The effectiveness of antibiotics depends on the type of antibiotics, the species of endosymbionts and the genetic group of *B. tabaci.* Further, the antibiotics used have various ranges of activity and modes of action. Tetracycline inhibits protein synthesis by blocking the binding of aminoacyl-tRNA in the A-site on the 30S bacterial ribosome (Jonas et al., 1984; Conte and Barriere, 1992; Costa et al., 1997; Ahmed et al., 2010).

On the basis of the results obtained it can be said that *Arsenophonus* free whiteflies have higher fitness than the *Arsenophonus* infected ones. These results point toward some negative effects of *Arsenophonus* infection in Asia II genetic group of *B. tabaci.* Such negative effects of secondary endosymbionts have been shown in other genetic groups (Q-biotype) of *B. tabaci* from China (Fang et al., 2014). Why *Arsenophonus* is widespread in populations of *B. tabaci* although being costly remains thus unclear. A potential mechanism of spread used by this symbiont may be to combine both vertical and horizontal transmission as reported for *Rickettsia* (Caspi-Fluger et al., 2012), *Arsenophonus* in parasitoid wasps (Skinner, 1985; Duron et al., 2010) and aphids (Jousselin et al., 2012): in these cases, the symbiont can spread in host populations without increasing host fitness.

Furthermore, while appreciating the results of the experiments based on bacterial endosymbiont elimination by antibiotic treatment, it should be borne in mind that such xenobiotic treatments would be having a broader effect (beyond the immediate targets like the endosymbionts- *Arsenophonus*, *Wolbachia* and *Rickettsia* in this case) affecting the diversity and prevalence of many gut bacteria. It could definitely be a possibility that the results obtained could be influenced by the changes in total metagenome, besides the obvious loss of *Arsenophonus* which could be easily detected. However, *Arsenophonus* being a major endosymbiont associated with diverse genetic groups of *B. tabaci*, being maternally transmitted to the offspring and it being completely eliminated from tetracycline treated parent and their F1 progeny lend support to the findings of this study that it might be responsible for altering the life history traits of *B. tabaci*.

In addition, *Arsenophonus* GroEl protein has been shown to be interacting with virus coat protein (Rana et al., 2012), suggesting an indirect role of *Arsenophonus* in virus transmission in spite of affecting the fitness of *B. tabaci*. The possible role of *Arsenophonus* in virus transmission needs further investigation.

Recently, Duron (2014) has reported association of a bacteriophage, APSE (*Acyrthosiphon pisum* secondary endosymbiont phage) with facultative endosymbiont *Arsenophonus* from different insect groups including whiteflies, aphids, parasitoid wasps, triatomine bugs, louse flies and bat flies. Further, Hansen et al. (2007) have reported the presence of a significant relation between variant infection frequency of *Arsenophonus* strain associated with APSE and the parasitism pressure in red lerp psyllid, *Glycaspis brimblecombei*. Furthermore, similarity of APSE associated with *Arsenophonus* (Hansen et al., 2007; Duron, 2014) has been reported to that of *Hamiltonella* (Moran et al., 2005; Degnan and Moran, 2008) involved in providing defence against parasitoids and other natural enemies.

5. Conclusion

This study revealed a complex relation between *Arsenophonus* and its host *B. tabaci*, wherein the endosymbiont lowered the fitness of its host. The reduction in different life-history parameters like fecundity, juvenile developmental time, nymphal survival and adult life span correlated to its presence in host affirmed the negative influence of this endosymbiont. However, despite this observation, these endosymbionts are still sustained in the host population, which raises a question about their functional role in *B. tabaci* and in other insect groups. More studies are needed to determine the physiological role of *Arsenophonus* in *B. tabaci.*

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